

DOD Protocol format with completion aids:

PROTOCOL TITLE: Title must include species of animal(s) used in research.

PRINCIPAL INVESTIGATOR(S)

CO-INVESTIGATOR(S)

I. NON-TECHNICAL SYNOPSIS: Provide a brief, narrative description of the proposal that is easily understood by a high school graduate. Include animal use in your description. (NOTE: This information may be used to complete the DOD Annual Report to Congress.)

II. BACKGROUND

II.1. Background: Include a brief statement of the requirement or need for the information being sought. Lengthy explanations are not required. Typically, the literature or the experience that led to the proposal will be briefly reviewed, references cited, and a description of the general approach will be provided.

II.2. Literature Search for Duplication: This search must be performed to prevent unnecessary duplication of previous experiments. A search of the Biomedical Research Database (BRD) is required. In addition, a search of EITHER the Federal Research in Progress (FEDRIP) OR the Computer Retrieval of Information of Scientific Projects (CRISP) database is required. Requirements for additional searches are at the discretion of the IACUC.

II.2.1. Literature Source(s) Searched

II.2.2. Date of Search

II.2.3. Period of Search

II.2.4. Key Words of Search

II.2.5. Results of Search: Provide a narrative description of the results of the literature search.

III. OBJECTIVE/HYPOTHESIS: State the objective of this protocol or the hypothesis to be accepted or rejected. (NOTE: This information will be used to complete the DOD Annual Report to Congress.)

IV. MILITARY RELEVANCE: Provide a brief and succinct military justification for the research with regard to military needs and mission requirements. If applicable, state the Science and Technology Objective (STO) that this work supports.

V. MATERIALS AND METHODS

V.1. Experimental Design and General Procedures: This section includes an explanation of experimental design. Technical methodology need not be described in this section, rather, it should be described under paragraph V.4, Technical Methods. Provide a complete description of the proposed use of animals to include a summary table of the experimental groups. Succinctly outline the formal scientific plan and direction of experimentation. If several experiments or sequential studies are to be included in the protocol, describe the experimental design of each separate experiment in sub-parts to this section. The length and detail required in this section depends largely on the complexity of the study. A clearly understandable description of the numbers of animals and their distribution into experimental groups is essential. The number requested must equal the minimum number required to complete the study yet be sufficient to yield meaningful results. The minimum number includes animals necessary for controls or technique development, and so forth. Inclusion of a summary table or flow chart showing the distribution of animals by experimental group is highly recommended. The total number of animals required for the study is listed in section V.3.4.

V.1.1. Experiment 1

V.1.2. Experiment 2

V.2. Data Analysis: List the statistical test(s) planned or describe the strategy intended to evaluate the data. Describe the statistical methodology used to determine group size and total number of animals. A power-based assessment of the sample size is the preferable method of determining the minimum number that is likely to yield significant results with given alpha and beta errors, estimated effect size and expected variability. Be certain to include animals necessary for controls or technique development, and so forth.

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered: State all non-animal alternatives (for example, computer modeling, in vitro cell culture work) that were considered. Explain why animals are needed.

V.3.2. Animal Model and Species Justification: Provide a scientific justification for the choice of animal model(s). What physiological and morphological characteristics does this animal possess that make it the best possible model? If less sentient (invertebrate versus vertebrate) animal models were considered but not chosen, explain why.

V.3.3. Laboratory Animals

V.3.3.1. Genus and Species

V.3.3.2. Strain/Stock: If inbred or specialized animals are required, use proper terminology. (See the attending veterinarian for assistance.)

V.3.3.3. Source/Vendor: Provide a preferred source for the animals. Animals will be legally obtained from suppliers licensed by the U.S. Department of Agriculture (USDA) in accordance with Code of Federal Regulations, Title 9, Animals and Animal Products, Chapter 1, Subchapter A, Animal Welfare, Parts 1, 2, and 3. (See the attending veterinarian for assistance.)

V.3.3.4. Age

V.3.3.5. Weight

V.3.3.6. Sex

V.3.3.7. Special Considerations: List specialized requirements for animals here (for example, simian immunodeficiency virus or herpes antibody free, Pasteurella free, and so forth).

V.3.4. Number of Animals Required (By Species): The number of animals stated here must correspond exactly to that described in section V.1. If, during the completion of the protocol, additional animals are needed owing to technical or unavoidable circumstances, or to exploit a serendipitous finding, follow IACUC procedures for requesting approval of additional animals.

V.3.5. Refinement, Reduction, Replacement (3 Rs): Investigators are required to consider the 3 Rs when preparing an animal use research protocol. In the paragraphs below, describe all provisions in this protocol that refine, reduce, or replace the use of animals. Discuss what provisions were considered and why they were not chosen. If N/A is used, explain why.

V.3.5.1. Refinement: Procedures or measures taken to eliminate or minimize pain or distress in the animal(s) or enhance animal well-being. Examples of refinement include but are not limited to the use of analgesia to decrease pain or distress, the use of remote telemetry, which decreases the distress of restraint, or the use of adjusted early experimental endpoints. In addition to listing refinements, list

refinement alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.3.5.2. Reduction: Procedures or measures taken to reduce the number of animals used. Examples of reduction include but are not limited to the use of shared or historical control groups, preliminary screening in non-animal systems, and innovative statistical packages. In addition to listing reductions that will be used, list reduction alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.3.5.3. Replacement: Procedures or measures that eliminate the use of animals. Examples of replacements include but are not limited to the use of non-animal models or less sentient animal species. In addition to listing replacements that will be used, also list replacement alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.4. Technical Methods: This information must be presented in sufficient detail, documented or referenced, so that the IACUC can adequately review the procedure, obtain a clear understanding of what is to be done and how the animals will be handled, and make a reasonable determination as to whether this proposed use of laboratory animals is in compliance with DOD regulations, guidelines, and Federal law.

V.4.1. Pain/Distress Assessment: The law defines a painful procedure as one that would "reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied; that is, pain in excess of that caused by injections or other minor procedures." If a procedure may involve pain or distress, even if relieved by anesthetics or analgesics, the P.I. must consult with the attending veterinarian.

V.4.1.1. APHIS Form 7023 Information: (See your attending veterinarian for assistance.) The protocol must contain an estimate of the number of animals that will be counted in columns C, D, and E of the APHIS Form 7023, Annual Report of Research Facility. Columns C, D and E represent specific pain categories. (See below paragraphs, V.4.1.1.1.-3.) The animal should be listed in the column corresponding to the most painful or distressful procedure experienced by the animal. It is possible for one protocol to have animals listed in several columns. For instance, control animals may be placed in Column C while experimental animals may be placed in Column D, depending upon the nature of the protocol. Reflect use of more than one species of animals in a duplicate table. The total numbers reflected in these three columns will add up to the number of animals requested for the entire protocol in paragraph V.3.4.

V.4.1.1.1. Number of Animals

V.4.1.1.1.1. Column C: __ (animal #)

Examples of research procedures/manipulations that would require an animal to be placed in Column C are studies involving not more than slight or momentary pain and/or distress in a human being to which that procedure is applied.

V.4.1.1.1.2. Column D: __ (animal #)

Examples of procedures/manipulations that would require an animal to be placed in Column D are procedures where anesthesia or analgesia will be administered to avoid or effectively relieve pain or distress. General anesthesia given for surgical procedures, or the use of analgesia or anti-inflammatory agents are examples of this category.

V.4.1.1.1.3. Column E: __ (animal #)

Examples of procedures/manipulations that would require an animal to be placed in Column E are procedures in which alleviation of pain or distress are contraindicated for a scientifically justifiable reason such as the experimental results are likely to be confounded if drugs relieving pain or distress were

administered. Detailed justification for putting animals into this category is required below in paragraph V.4.1.4.

V.4.1.2. Pain Relief/Prevention

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization: Describe the methods or strategies planned to effectively relieve or prevent pain or distress if the study will cause more than slight or momentary pain or distress. If pain/distress relief/prevention is planned, specify agents to be used and when these agents will be given (pre-emptive or post-procedural). Provide agent, dosage, and frequency of administration.

V.4.1.2.2. Pre- and Post-procedural Provisions: Describe the provisions for both pre- and post-procedural care, including provisions for post-procedural observations and frequency of observations. (Information concerning pre- and post-surgical care should be listed in paragraphs V.4.3.1 and V.4.3.3). If analgesics are used for pain/distress relief, provide the frequency of administration, observational criteria utilized to determine if animals are experiencing pain or distress, and the location for the post-procedural care.

V.4.1.2.3. Paralytics: The use of paralytic agents without anesthesia is prohibited. Describe the monitoring method that will be used to ensure adequate depth of anesthesia while the animal is under the influence of the paralytic agent.

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures: Respond N/A if the animals will experience not more than momentary or slight pain or distress and are placed in column C of APHIS Form 7023. (See paragraph V.4.1.1.)

V.4.1.3.1. Source(s) Searched: Examples are AGRICOLA, MEDLINE, BIOSIS, Altweb, and so forth.

V.4.1.3.2. Date of Search

V.4.1.3.3. Period of Search

V.4.1.3.4. Key Words of Search: Examples are pain, surgery, alternatives, LD 50, analgesia, anesthesia, death as an endpoint, distress, species of animal(s) to be used, name of painful or distressful experimental procedure, and so forth.

V.4.1.3.5. Results of Search: Provide a narrative summary of the results of the literature search for alternatives. The Animal Welfare Act specifically states that the P.I. must provide a narrative description of the methods and sources, e.g., the Altweb (Johns Hopkins Center for Alternatives to Animal Testing), MEDLINE, Life Sciences Abstracts, AGRICOLA, and BIOSIS) that he/she used to determine that alternatives to the painful procedure were not available. Discuss alternatives (those that would meet your scientific objectives) considered but not chosen. The alternatives literature search MUST be performed even when animals are placed in Column D and the pain or distress is alleviated through the use of analgesics or anesthetics.

V.4.1.4. Unalleviated Painful/Distressful Procedure Justification: Procedures that cause more than slight or momentary pain or distress that is not alleviated through the effective use of anesthetics or analgesics must be justified on a scientific basis in writing by the P.I. This paragraph must be completed if there are ANY animals in this protocol that will experience unalleviated pain or distress.

V.4.2. Prolonged Restraint: Describe (period of restraint, method, and timing of animal observations, habituation/training of animal to restraint device) and justify in detail any prolonged restraint greater than 12 hours for nonhuman primates or in accordance with IACUC policy for other species. Examples of restraint methods are primate chairs, restraint boards, metabolism cages, and so forth. This section is not intended for short-term actions such as rabbit restraint for bleeding, and so forth.

V.4.3. Surgery: Major survival operative procedures on non-rodent species will be conducted only in dedicated facilities intended for that purpose, and operated and maintained under aseptic conditions.

Non-survival operative procedures do not require a dedicated facility, but they should be performed using surgical gloves, mask, and clean instruments. Additionally, the surgical site should be clipped and cleaned prior to surgery. Major survival rodent surgery does not require a dedicated facility but it must be performed using aseptic technique; that is, aseptic patient preparation, surgical gloves, mask, and sterile instruments. A major operative procedure is defined as a procedure that penetrates and exposes a body cavity, or causes substantial or permanent impairment of physical or physiological function.

V.4.3.1. Pre-Surgical Provisions: Describe the provisions for pre-surgical care, including provisions for pre-surgical observations and frequency of pre-surgical observations. If analgesics are utilized for pain or distress relief, provide the time schedule for administration, observational criteria utilized to determine if animals are experiencing pain/distress, and the location for the pre-surgical care.

V.4.3.2. Procedure: Describe in detail any surgical procedures planned.

V.4.3.3. Post-Surgical Provisions: Describe the provisions for post-surgical care, including provisions for post-surgical observations, frequency of post-surgical observations and criteria for early euthanasia owing to surgical complications or pain that cannot be relieved. If analgesics are utilized for pain or distress relief, provide the time schedule for administration, observational criteria utilized to determine if animals are experiencing pain/distress, and the location for the post-surgical care.

V.4.3.4. Location: Give the location/room number for the proposed surgical procedure.

V.4.3.5. Surgeon

V.4.3.6. Multiple Major Survival Operative Procedures: The principal investigator must scientifically justify multiple major survival operative procedures performed on the same animal.

V.4.3.6.1. Procedures

V.4.3.6.2. Scientific Justification

V.4.4. Animal Manipulations: Describe any injections, sampling procedures, or other manipulations of the animals necessary for the study. A reference or SOP may be furnished to the IACUC to document a particular procedure in lieu of a detailed description.

V.4.4.1. Injections: Information must include route of injection, dosage, frequency, volume injected, needle size, and anatomic injection site.

V.4.4.2. Biosamples: Examples include cerebrospinal fluid taps, blood sampling, and biopsies. List volumes taken, sampling site, frequency of sampling, needle size, and method of sampling. Procedures performed or biosamples obtained during a necropsy need not be described here.

V.4.4.3. Adjuvants: List any adjuvants used and the plan for their use. Provide a scientific justification for the use of Complete Freund's Adjuvant (CFA) and discuss why other less reactive adjuvants cannot be used. Provide dosages, volumes, route, number of injection sites, and injection locations. Specify frequency and method of injection site monitoring and include a response plan (for example, alternative endpoint and veterinary medical treatment) in the event of an adverse reaction.

V.4.4.4. Monoclonal Antibody (MAbs) Production: Provide a scientific justification for in vivo MAbs production. What in vitro methods of MAbs production were considered but not used? For in vivo MAbs production, specify the priming agent, animal monitoring frequency, number and frequency of abdominal taps, and fluid replacement therapy. Include a response plan (for example, alternative endpoint and veterinary medical treatment) in the event of an adverse reaction.

V.4.4.5. Animal Identification: Describe the method of animal identification used in this study. Examples include microchips, tattoos, ear tags, and cage cards.

V.4.4.6. Behavioral Studies: Fully describe the use of aversive stimuli, food or water restriction, and so forth, that would affect the study animals. Include methods of monitoring physiologic or behavioral indexes, including criteria (for example, weight loss or state of hydration) for temporary or permanent removal of the animal from the study. Provide an appropriate scientific justification for this type of behavior modification. An IACUC policy may be included where applicable.

V.4.4.7. Other Procedures: Describe all procedures which have not been explained in other sections of this proposal that will be performed while conducting this research. Examples include electrocardiograms, radiology, and aerosol exposure.

V.4.4.8. Tissue Sharing: List what tissues will be shared, with whom, and for what purpose.

V.4.5. Study Endpoint: State the projected study endpoint for the animals (for example, recovery and return to issue pool, euthanasia, or death without early euthanasia). Indicate whether recovery, euthanasia, or death is expected; and the specific plan for determining when the animal experimentation phase will be stopped. The P.I. must ensure that unnecessary pain or distress is prevented by carefully considering "When is the experimental question answered?" so that the animals can be expeditiously removed from the study. Define specific criteria that will be used to determine study endpoint (for example, weight loss, loss of locomotion and significant lowering of body temperature, decreased food or water consumption, and decreased activity). Specifically address and scientifically justify any proposal in which critically ill or moribund animals are allowed to die as a result of the experimental procedures without the benefits of veterinary medical treatment or early euthanasia. Explain the plan for the disposition of surviving animals or animals removed from the study prior to its completion.

V.4.6. Euthanasia: If applicable, discuss the euthanasia method. The Animal Welfare Act defines euthanasia as "humane destruction of an animal by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death." The current American Veterinary Medical Association (AVMA) guidelines for euthanasia must be followed. Exceptions to the AVMA guidelines will be considered by the IACUC on a case-by-case basis. If requested, the attending veterinarian will assist in selecting the best method for euthanasia.

V.5. Veterinary Care: If requested, the attending veterinarian of the facility will assist PIs with preparing this section.

V.5.1. Husbandry Considerations: Federal regulations require that animal housing and living conditions must be appropriate to their species and contribute to their health and comfort. Briefly describe animal husbandry to include routine animal observations, caging methods, feed and water provisions, environmental parameters, sanitation schedules, and light cycles.

V.5.1.1. Study Room: Where will the experimental procedure be conducted? Will the animal be housed in this room for more than 12 hours?

V.5.1.2. Special Husbandry Provisions: Examples include micro-isolators, metabolic cages, food and water restriction.

V.5.1.3. Exceptions: Describe any deviations/exceptions to *The Guide for the Care and Use of Laboratory Animals*, the Animal Welfare Act regulations, or IACUC policy that have an impact on animal housing space, feeding, and sanitation. Deviations/exceptions must be justified by the P.I. and approved by the IACUC.

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care: Describe the routine veterinary medical care. State if the animals will be observed daily or more frequently. Indicate what will happen if the animal becomes ill or

debilitated during the study and requires evaluation. List the criteria used for health evaluation while the animals are on study (for example, weight loss, ruffled fur, dehydration, decreased activity, and hunched body position). Include a response plan (for example, alternative early endpoint and veterinary medical treatment) in the event of debilitating illness or an adverse reaction.

V.5.2.2. Emergency Veterinary Medical Care: Describe emergency veterinary medical care.

V.5.3. Environmental Enrichment

V.5.3.1. Enrichment Strategy: Discuss enrichment provided to animal species listed in this protocol.

V.5.3.2. Enrichment Restriction: Provide written justification for restricting enrichment programs or activity programs of dogs, cats, or nonhuman primates. Single housing of nonhuman primates and dogs without sensory contact with conspecifics must also be justified and approved by the IACUC.

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING: List the names, qualifications and training by procedure of all personnel working with animals assigned to this protocol. Personnel performing observations, procedures, and/or manipulations described in the protocol must be identified and appropriately trained and qualified to perform these procedures. Contact the attending veterinarian for assistance with this requirement.

VII. BIOHAZARD/SAFETY: Provide a list of any potential biohazards associated with the chosen animal model and this research proposal (for example, viral agents, toxins, radioisotopes, oncogenic viruses, and chemical carcinogens). Describe safety precautions and programs designed to protect personnel from biohazards associated with this research and any surveillance procedures in place to monitor potential exposures.

VIII. ENCLOSURES: Enclosures such as IACUC policies on adjuvants, monoclonal antibody production, tissue sharing, food and/or water restriction, prolonged restraint, pathology addenda, and pain assessment criteria may be included at the discretion of the PI unless directed by the IACUC.

C-5. Personnel qualifications.

a. A Study Personnel Qualifications/Training table must be included in section VI of the protocol description. The table format is preferred by the IACUC for ease of reviewing the protocol. The table will contain the following four column headings:

- (1) Name of the activity (for example, the procedure, observation, or manipulation to be performed, such as the venous catheterization of a dog).
- (2) Name of the person performing the activity.
- (3) Qualifications of the person performing the activity (for example, assistant laboratory animal technician (ALAT), 2 years experience).
- (4) Training of the person performing the activity (for example, Canine Procedures Workshop, 1999).